

In the Claims.

Please amend the claims as follows:

1. (Cancelled)
2. (Currently Amended) The ~~PCR reaction mixture of claim 1 method of claim 24~~ wherein the dsDNA binding dye has a percent saturation of at least 80%.
3. (Currently Amended) The ~~PCR reaction mixture of claim 1 method of claim 24~~ wherein the dsDNA binding dye has a percent saturation of at least 90%.
- 4-16. (Cancelled)
17. (Currently Amended) ~~A method of genotyping comprising the steps of:~~
~~amplifying a target nucleic acid in the presence of a dsDNA binding dye having a percent saturation of at least 50% The method of claim 24 wherein the monitoring step comprises~~
melting the amplified target nucleic acid to generate a melting curve, and identifying the genotype ~~from~~ using a shape of the melting curve.
18. (Currently Amended) The method of ~~claim 17~~ claim 24 wherein the ~~melting curve is generated monitoring step is performed~~ using a fluorimeter having an excitation range of 450-490 nm and an emission detection range of 510-530 nm, and the dye has an excitation maximum in a range of 410-465 nm and an emission maximum in a range of 450-500 nm.
19. (Original) The method of claim 18 wherein the dye's excitation maximum is in the range of 430-460 nm and emission maximum is in the range of 450-500 nm.
20. (Original) The method of claim 17 wherein the target nucleic acid comprises a single nucleotide polymorphism, and the identifying step comprises identifying resultant heteroduplexes and homoduplexes.
- 21-22. (Cancelled)
23. (Currently Amended) ~~A method of mutation scanning comprising the steps of:~~

- (a) — adding a dsDNA binding dye having a percent saturation of at least 50% to a sample comprising a target nucleic acid,
- (b) — amplifying the target nucleic acid in the presence of the dsDNA binding dye, (c)

The method of claim 24 wherein the method comprises mutation scanning, the monitoring step comprises melting the amplified target nucleic acid to generate a melting curve, and the method further comprises

- (d) — repeating the amplifying and monitoring steps (b) and (e) on second sample to obtain a second melting curve, and

- (e) — comparing the melting curves.

24. (Original) A method of PCR analysis comprising the steps of:

mixing a dsDNA binding dye having a percent saturation of at least 50% with a sample comprising a target nucleic acid and primers configured for amplifying the target nucleic acid,

amplifying the target nucleic acid in the presence of the dsDNA binding dye, and

monitoring fluorescence of the dsDNA binding dye.

25. (Original) The method of claim 24 further comprising the steps of

generating a melting curve for the target nucleic acid,

normalizing the magnitude of the melting curve,

repeating the mixing, amplifying, generating, and normalizing steps with at least one additional target nucleic acid, and

comparing the normalized melting curves.

26. (Currently Amended) The method of claim 25 further comprising the step of plotting the ~~temperature~~ fluorescence difference between the normalized curves.

27. (Original) The method of claim 25 further comprising the step of temperature shifting the melting curves by superimposing a portion of each curve.

28. (Currently Amended) The method of claim 27 further comprising the step of plotting the ~~temperature~~ fluorescence difference between the temperature shifted curves.

29. (Currently Amended) The method of claim 24 or 25 wherein the dye is selected from the group consisting of LC Green, Gel Star, PO-PROTM-1, JO-PROTM-1, and BO-PROTM-1, and SYTO[®] 16.

30. (Currently Amended) The method of claim 25 wherein the dye is selected from the group consisting of PO-PROTM-1, JO-PROTM-1, BO-PROTM-1, SYTO[®] 44, SYTO[®] 45, YO-PRO[®]-1, POPOTM-3, SYTO[®] 12, TOTOTM-3, SYTOX[®] Blue, Thiazole Orange, YOYO[®]-3, SYTO[®] 43, SYTO[®] 11, SYTO[®] 13, SYTO[®] 15, BOBOTM-3, LO-PROTM-1, SYTO[®] 23, TO-PRO[®]-1, SYTO[®] 20, BOBOTM-1, POPOTM-1, G5, H5, D6, E6, P6, R6, Y6, Z6, and D8.

31-32. (Cancelled)

33. (Original) The method of claim 24 wherein the sample further comprises a probe configured to hybridize to the target nucleic acid, said probe labeled with an acceptor dye to accept fluorescent resonance energy transfer from the dsDNA binding dye, and further comprising the step of monitoring fluorescence from the acceptor dye.

34. (Currently Amended) The method of ~~claim 24~~ claim 17 wherein the target nucleic acid is no greater than 100 bp.

35. (Currently Amended) The method of ~~claim 34~~ claim 17 wherein the target nucleic acid is no greater than ~~50~~ 61 bp and comprises only a single melting domain.

36. (Cancelled)

37. (Currently Amended) The method of ~~claim 36~~ claim 25 wherein the target nucleic acid comprises a variable melting domain and an invariant melting domain and the method further comprising comprises the steps of

generating a melting curve for the target nucleic acid,

repeating the mixing, amplifying and generating steps with at least one additional target nucleic acid,

using the invariant melting domain for temperature axis adjustment, and

comparing the melting curve for the target nucleic acid with the melting curve for the additional target nucleic acid.

38. (Cancelled)

39. (Original) The method of claim 24 wherein the amplifying and monitoring occur in a closed tube, and no reagents are added to the tube subsequent to initiation of amplification.

40. (Original) The method of claim 24 wherein the monitoring step occurs subsequent to the amplifying step and comprises melting curve analysis.

41. (Original) The method of claim 24 wherein the monitoring step occurs during amplification.

42. (Original) The method of claim 41 further comprising the step of performing post-amplification melting curve analysis.

43. (Currently Amended) ~~A method of PCR analysis comprising cycling a PCR mixture of claims 1-17 between at least an annealing temperature and a denaturation temperature to amplify the target nucleic acid,~~

The method of claim 24 wherein the monitoring step comprises
generating a melting curve for the target nucleic acid, and
using the melting curve to determine whether the target nucleic acid has the same sequence as a second nucleic acid.

44. (Cancelled)

45. (Original) A method of PCR analysis comprising the steps of:
mixing a dsDNA binding dye with a sample comprising a target nucleic acid and primers configured for amplifying the target nucleic acid,
amplifying the target nucleic acid in the presence of the dsDNA binding dye,
monitoring fluorescence of the dsDNA binding dye,
generating a melting curve for the target nucleic acid,
normalizing the melting curve,
repeating the mixing, amplifying, normalizing, and generating steps with at least one additional target nucleic acid, and
comparing the normalized melting curves.

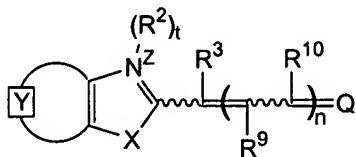
46. (Currently Amended) The method of claim 45 further comprising the step of plotting the temperature fluorescence difference between the normalized curves.

47. (Original) The method of claim 45 further comprising the step of

temperature shifting the melting curves by superimposing a portion of each curve.

48. (Currently Amended) The method of claim 47 further comprising the step of plotting the ~~temperature~~ fluorescence difference between the temperature shifted curves.

49. (Currently Amended) A compound having the formula:



wherein

the moiety Y represents an optionally-substituted fused monocyclic or polycyclic aromatic ring or an optionally-substituted fused monocyclic or polycyclic nitrogen-containing heteroaromatic ring;

X is oxygen, sulfur, selenium, tellurium or a moiety selected from C(CH₃)₂ and NR¹, where R¹ is hydrogen or C₁₋₆ alkyl;

R² is selected from the group consisting of C₁₋₆ alkyl, C₃₋₈ cycloalkyl, aryl, aryl(C₁₋₂ alkyl), hydroxyalkyl, alkoxyalkyl, aminoalkyl, mono and dialkylaminoalkyl, trialkylammoniumalkyl, alkylenercarboxylate, alkylenercarboxamide, alkylenesulfonate, alkylsulfonate, optionally substituted cyclic heteroatom-containing moieties, and optionally substituted acyclic heteroatom-containing moieties;

t = 0 or 1;

Z is a charge selected from 0 or 1;

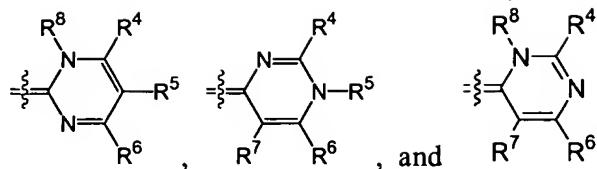
~~R³, R⁹, and R¹⁰ are each independently selected from the group consisting of hydrogen and C₁₋₆ alkyl;~~

R³ is selected from the group consisting of hydrogen, C₁₋₆ alkyl, and -C(O)Ph;

R⁹ and R¹⁰ are each independently selected from the group consisting of hydrogen and C₁₋₆ alkyl;

n = 0, 1, or 2; and

Q is an heterocycle selected from the group of structures consisting of:



wherein R⁴, R⁵, R⁶, R⁷, and R⁸ are independently selected from the group consisting of hydrogen, halogen, alkyl, cycloalkyl, heteroalkyl, heterocycloalkyl, alkenyl, polyalkenyl, alkynyl, polyalkynyl, alkenylalkynyl, aryl, heteroaryl, alkoxy, alkylthio, and dialkylamino, each of which may be optionally substituted; an acyclic heteroatom-containing moiety or a cyclic heteroatom-containing moiety; a BRIDGE-DYE; and a reactive group; each of which optionally includes a quaternary ammonium moiety.

50. (Currently Amended) The compound of claim 49 wherein the moiety Y represents an optionally-substituted fused monocyclic or polycyclic aromatic ring selected from the group consisting of optionally substituted benzo, optionally substituted pyridino, and optionally substituted naphtho; and X is oxygen or sulfur.

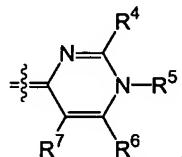
51. (Original) The compound of claim 49 wherein the moiety Y represents a benzo or a naphtho having a substituent selected from the group consisting of halo, alkyl, amino, monoalkylamino, dialkylamino, alkylsulfonyl, haloalkylsulfonyl, and optionally substituted phenylsulfonyl.

52. (Cancelled)

53. (Currently Amended) The compound of claim 49 wherein R² is selected from the group consisting of C₁₋₆ alkyl, C₃₋₈ cycloalkyl, aryl, aryl(C₁₋₂ alkyl), aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl, trialkylammoniumalkyl, alkylsulfonate, alkylenesulfonate, optionally substituted cyclic heteroatom-containing moieties, and optionally substituted acyclic heteroatom-containing moieties.

54. (Cancelled)

55. (Original) The compound of claim 49 wherein Q is the heterocycle:



56. (Currently Amended) The compound of claim 49 wherein R⁴, R⁵, R⁶, R⁷, and R⁸ are independently selected from the group consisting of hydrogen, halogen, thiol,

alkylthio, alkyl, aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl, trialkylammoniumalkyl, piperidino, piperazino, 4-methylpiperazinium-1-yl, and aryl.

57. (Currently Amended) The compound of claim 49 wherein t is 1, n = 0, and at least one of R⁴, R⁵, R⁶, R⁷, and R⁸ is selected from the group consisting of halogen, thiol, alkylthio, C₂₋₆ alkyl, aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl, trialkylammoniumalkyl, piperidino, piperazino, 4-methylpiperazinium-1-yl, and aryl.

58. (Original) The compound of claim 57 wherein R⁵ is selected from the group consisting of halogen, thiol, C₂₋₆ alkyl, aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl, trialkylammoniumalkyl, piperidino, piperazino, 4-methylpiperazinium-1-yl, and aryl.

59-60. (Cancelled)

61. (Currently Amended) The compound of claim 57 wherein R³, R⁹, and R¹⁰ are each hydrogen; and R² is selected from the group consisting of C₁₋₆ alkyl, aryl, aryl(C₁₋₂ alkyl), aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl, trialkylammoniumalkyl, alkylsulfonate, and alkylenesulfonate.

62-64. (Cancelled)

65. (Currently Amended) The method of ~~claim 63~~ claim 43, wherein the target nucleic acid is a locus of an HLA gene from a first individual and the second nucleic acid is the same locus of an HLA gene from a second individual.

66. (Original) The method of claim 65 wherein the melting curve for the target nucleic acid is similar to the melting curve for the second nucleic acid, and further comprising the steps of

generating a melting curve for a mixture of the target nucleic acid and the second nucleic acid, and

comparing the melting curve for the target nucleic acid or the second nucleic acid with the melting curve for the mixture of the target nucleic acid and the second nucleic acid.

67-82. (Cancelled)